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Virus Research 102 (2004) 215–220
Virus Research
www.elsevier.com/locate/virusres

Seven new ovine progressive pneumonia virus (OPPV) field isolates from Dubois Idaho sheep comprise part of OPPV clade II based on surface envelope glycoprotein (*SU*) sequences

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Received 25 October 2003; received in revised form 3 February 2004; accepted 3 February 2004

Abstract

Seven new ovine progressive pneumonia virus (OPPV) field isolates were derived from colostrum and milk of 10 naturally OPPV-infected sheep from the US Sheep Experiment Station in Dubois, Idaho, USA. Sixteen sequences of the surface envelope glycoprotein (*SU*) from these seven Dubois OPPV field isolates and *SU* sequence from OPPV WLC1 were obtained, aligned with published SRLV *SU* sequences, and analyzed using phylogenetic analysis using parsimony (PAUP). Percent nucleotide identity in *SU* was greater than 95.8% among clones from individual Dubois OPPVs and ranged from 85.5 to 93.8% between different Dubois OPPV clones. *SU* sequences from Dubois OPPVs and WLC1 OPPV had significantly higher percent nucleotide identity to *SU* sequences from the North American OPPVs (85/34 and S93) than caprine-arthritis encephalitis virus (CAEVs) or MVVs. PAUP analysis also showed that *SU* sequences from the Dubois OPPVs and OPPV WLC1 grouped with other North American OPPVs (85/34 and S93) with a bootstrap value of 100 and formed one OPPV clade II group. In addition, Dubois and WLC1 *SU* amino acid sequences had significantly higher identity to *SU* sequences from North American OPPVs than CAEV or MVV. These data indicate that the seven new Dubois OPPV field isolates along with WLC1 OPPV are part of the OPPV clade II and are distinct from CAEVs and MVVs.
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Keywords: SRLV; OPPV; CAEV; MVV; Colostrum; Milk; Envelope; *SU*

1. Introduction

Small ruminant lentiviruses (SRLVs) include caprine-arthritis encephalitis virus (CAEV), maedi-visna virus (MVV), and ovine progressive pneumonia virus (OPPV), the North American equivalent to MVV. SRLVs have been categorized based on cytopathogenicity in cell culture (Querat et al., 1984; Lairmore et al., 1987). Currently, SRLVs are being categorized according to their nucleotide sequence. Nucleotide sequence information establishes the phylogenetic relationship between different SRLVs. In SRLV research, one study recently grouped SRLVs into clades according to partial and complete *env* sequences (Zanoni, 1998).

OPPV was first reported in North America in sheep from Montana, USA experiencing respiratory distress or also known as "lunger" sheep (Marsh, 1923). The presence of respiratory distress in the absence of any central nervous system disorders or visna defined the respiratory viral infection as OPPV. A few OPPV field isolates have been reported over the last 35 years in North America and most have been isolated from respiratory distress or maedi clinical cases. The OPPV isolate (CU1) presently named 85/34 was derived from a sheep experimentally inoculated with an isolate derived from lung tissue of white face Montana sheep with clinical signs of OPPV (Lairmore et al., 1987;

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doi:10.1016/j.virusres.2004.02.001

Kennedy et al., 1968). The WLC1 OPPV isolate was originally isolated from lung tissue of Wyoming sheep with clinical signs of OPPV (Cutlip and Laird, 1976), and the S93 OPPV isolate was originally isolated from a Border Leichester sheep in Maryland with clinical signs of OPPV (Jolly and Narayan, 1989). Another OPPV isolate was derived from lung of a Finnish ewe in upstate New York with clinical signs of OPPV (Campbell et al., 1993). Twenty-four years have past without any new information regarding OPPV in the Rocky Mountain region of USA where the highest OPPV seroprevalence occurs (Cutlip et al., 1992). Evaluation of new SRLVs will enable a better understanding of SRLV ancestral origins and their transmissibility between species.

OPPV is believed to be transmitted via colostrum/milk and/or respiratory secretions. In this study, seven new OPPV field isolates were derived from colostrum or milk of 10 lactating OPPV-positive ewes originating from the US Sheep Experiment Station in Dubois, Idaho. Sixteen new *SU* sequences were obtained from these seven new OPPV field isolates. *SU* sequence was also obtained from the OPPV WLC1, currently used as the antigen for serologic diagnosis employing the agar gel immunodiffusion assay (AGID) (Cutlip et al., 1977). *SU* sequence from the Dubois OPPV isolates and WLC1 OPPV were analyzed against *SU* sequences from known SRLVs.

2. Materials and method

2.1. Animals

Ten pregnant naturally OPPV-infected sheep not experiencing clinical signs of maedi were moved from their original location of Dubois, Idaho to Pullman, WA. All sheep were white-faced sheep composed of Polypay, Rambouillet, and Columbia breeds. Their OPPV-infection status was determined by the presence of anti-surface envelope glycoprotein antibodies detected by competitive-inhibition enzyme-linked immunosorbent assay (cELISA) and immunoprecipitation of ^{35}[S] methionine-labeled OPPV WLC1 lysates (Herrmann et al., 2003b).

2.2. Virus isolation from co-culture and DNA isolation

Within 24 h after parturition, 10–30 ml of colostrum was collected by milking. Briefly, the ewe's teat was cleaned with cotton gauze soaked in 70% ethanol, dried for approximately 2 min followed by milking. Every 2 weeks, 10–30 ml of milk from the 10 Dubois ewes was also collected. Colostrum and milk was diluted 1:1 with 1 × PBS Dubellco's pH 8.0 and cells were recovered by centrifugation at 500 × *g* for 10 min at 4 °C. Cells were washed 2× with DMEM 10% fetal bovine serum (FBS) with 100 μg/ml gentamycin, and 100 U/ml penicillin/streptomycin, suspended into 1 ml media and split between 2 and 25 cm^2 flasks containing goat synovial membrane cells (GSM) at approximately 50% confluence. The co-cultures were maintained in DMEM 5.0% FBS with 100 μg/ml gentamycin, 100 U/ml penicillin/streptomycin.

The cells were passed as needed and observed for 6 weeks for formation of syncytia. When syncytia were observed, the supernatant was placed on fresh GSM cells and followed 6 more weeks to verify the presence of syncytia. The syncytia-forming co-cultured cells were trypsinized and collected by centrifugation at 500 × *g* for 10 min at 4 °C and frozen at −20 °C until DNA isolation could be performed. OPPV strain WLC1 was grown in GSM cells and upon the presence of syncytia, cells were collected for DNA isolation. DNA from co-cultured colostral/milk and GSM cells was isolated using a DNA isolation kit for 30–50 × 10^6 cultured cells (Gentra Puregene D-5000K).

2.3. PCR amplification, cloning, and sequencing of SU

PCR amplification of the envelope gene was performed on DNA (100–500 ng) using the following reagents: $[0.0375\ \text{U}/\mu\text{l}]_\text{f}$ platinum Taq polymerase (Invitrogen), $1 \times \text{PCR}_\text{f}$ Mix (Invitrogen), $[1.5\ \text{mM}]_\text{f}$ MgCl_2 (Invitrogen), $[0.2\ \text{mM}]_\text{f}$ dNTPs (Invitrogen), and $[3\ \text{ng}/\mu\text{l}]_\text{f}$ of the forward and reverse primers. The forward primer for WLC-1 and sheep LMH 17 was TATF3: 5′-TGTGGATGCAGACTATGTAACCCGGGGTGGGGAA-3′. TATF3 was derived from CAEV-CO (Saltarelli et al., 1990). The forward primer for sheep LMH 11, 13, 15, 16, 18, and 19 was MV-TATF1: 5′-TGTGGGTGTCGACTATGCAACCCGGGATGGGGAA-3′. The reverse primer for WLC-1 was WLCR1: 5′-CTGTCTAAAGTAGCATTTTTGCA-3′. The reverse primer for LMH 11, 13, 15, 16, 17, and 19 was S93R: 5′-CTCATCCTGCTGNGCAGCAGTTGC-3′. MV-TATF1 and S93R were designed based upon Genbank sequences of OPPV 85/34 (AY101611) and OPPV S93 (AF338226). PCR amplification conditions were as follows: 1 cycle at 95 °C for 4 min; 35 cycles of the following: 95 °C for 30 s, 50 °C for 30 s, and 72 °C for 2 min; 1 cycle of 72 °C for 7 min; 4 °C indefinite. PCR products were analyzed in an ethidium bromide 1% low melt agarose gel, bands at 2 kbp were excised, and DNA was isolated from excised gel fragments using the Qiaex II agarose gel extraction protocol (Qiagen). DNA was quantified by $A_{260\ \text{nm}}$ and an extension reaction is performed at 72 °C for 20 min using $[1 \times \text{PCR}]_\text{f}$, $[0.3\ \text{mM}]_\text{f}$ dNTPs, $[1.5\ \text{mM}]_\text{f}$ MgCl_2, and $[0.1\ \text{U}/\mu\text{l}]_\text{f}$ platinum Taq polymerase (Invitrogen). Extended DNA was cloned into pCR2.1 using TOPO TA Cloning Kit (Invitrogen), and TOP 10 cells (Invitrogen) were transfected with pCR2.1. DNA was isolated from 5 ml cultures of individually picked colonies using an SV Mini-Prep Kit (Promega). Clones with the envelope insert were identified by EcoRI digest at $[2\ \text{U}/\mu\text{l}]_\text{f}$ for 2 h at 37 °C followed by analysis on a 1% agarose gel containing ethidium bromide. Positive envelope clones were sequenced using a fluorescent dideoxy-terminator sequencing method at Washington State University Biomolecular Core Sciences

Laboratory using ABI Prism 337 or ABI 3100 DNA sequencers.

2.4. Alignment and phylogenetic analysis of SU

Nucleotide sequences encoding the surface envelope glycoprotein of the clones from WLC1, LMH 11, 13, 15, 16, 17, 18, and 19 were assembled and analyzed using Seqman Dnastar software (Lasergene). We obtained more than one clone in six out of the seven new OPPV isolates yielding a total of 16 new OPPV *SU* nucleotide sequences. *SU* nucleotide sequences of the Dubois OPPVs, WLC1 OPPV, and the SRLV *SU* nucleotide sequences listed in Table 1 were aligned using ClustalW in MegAlign Dnastar software (Lasergene). Aligned *SU* nucleotide sequences were exported in phylogenetic analysis using parsimony (PAUP) format to PAUP version 4.0 for phylogenetic tree construction. A parsimony with heuristic phylogenetic tree was constructed using bovine immunodeficiency virus (BIV) *env* (Genbank accession number M32690) as the outgroup and bootstrap confidence values were calculated based on 1000 iterations. In addition, deduced amino acid *SU* sequences were completed for the Dubois OPPVs, WLC1 OPPV, and the SRLV strains listed in Table 1 using Editseq Dnastar software (Lasergene), and were aligned using ClustalW. The MVV *SU* consensus was determined by ClustalW alignment of *SU* from the MVVs listed in Table 1. The OPPV *SU* consensus was determined by ClustalW alignment of *SU* from WLC1 OPPV, 85/34 OPPV, and S93 OPPV. The CAEV *SU* consensus was determined by ClustalW alignment of *SU* from the CAEVs listed in Table 1 including CAEV 1244.

Genbank accession numbers AY362022 thru AY362038 were assigned to the 16 *SU* nucleotide sequences obtained from the Dubois OPPVs and the one *SU* nucleotide sequence from WLC1 OPPV.

Table 1
SU sequences analyzed in this study from the SRLV strains listed along with their Genbank accession numbers and corresponding references

SRLV Strain	Genbank accession number	References
OPPV 85/34	U64439	Woodward et al., 1995
OPPV S93	AF338226	Hötzel and Cheevers, 2001
CAEV-63	M60855	Knowles et al., 1991
CAEV-CO	M33677	Saltarelli et al., 1990
CAEV-680	AF015180	Valas et al., 1997
CAEV-021	AJ400719	Valas et al., 2000
CAEV-032	AJ400720	Valas et al., 2000
CAEV-786	AJ400721	Valas et al., 2000
MVV-EV1	S51392	Sargan et al., 1991
MVV-KM1071	U51910	Andresdottir et al., 1998
MVV-1772	L06906	Braun et al., 1987
MVV-SA-OMVV	M31646	Querat et al., 1990
MVV-1514-LV1-1KS1	M60609; M37977	Staskus et al., 1994

3. Results and discussion

3.1. Percent nucleotide identity in SU of Dubois OPPV field isolates

Seven new OPPV field isolates were derived from the colostrum or milk cell co-cultures with goat synovial membrane (GSM) cells. Proviral DNA was isolated from the co-cultured cells that produced syncytia either within 6 weeks of the initial co-culture or after 6 weeks of passing the first supernatant onto fresh GSM cells. The envelope gene was PCR amplified from the proviral DNA, cloned and sequenced. The seven new OPPV field isolates resulted in a total of 16 new OPPV *SU* nucleotide sequences. Percent nucleotide identity in *SU* was greater than 95.8% among clones from individual Dubois OPPVs and ranged from 85.5 to 93.8% between different Dubois OPPV clones.

3.2. Percent nucleotide identity in SU between SRLVs

SU sequences obtained from WLC1 OPPV and Dubois OPPVs were compared to *SU* sequences from several published SRLVs listed in Table 1. Table 2 shows the mean percent nucleotide identity in *SU* of the 16 new OPPV Dubois *SU* sequences and the one WLC1 *SU* sequence compared to WLC1 OPPV, 85/34 OPPV, S93 OPPV, CAEV, and MVV *SU* sequences. *SU* of WLC1 OPPV had a higher percent identity to 85/34 OPPV *SU*, S93 OPPV *SU*, and Dubois OPPV *SU* than to CAEV or MVV *SU* sequences (Table 2). In addition, the Dubois *SU* sequences had a significantly higher mean percent nucleotide identity ±2 standard deviations (2 S.D.) to OPPV WLC1, 85/34, and S93 *SU* sequences than to CAEV or MVV *SU* sequences (Table 2). Although it was not significant, the Dubois *SU* sequences had slightly higher mean percent nucleotide identity to CAEV *SU* sequences (74.4%) than MVV *SU* sequences (71.7%). Four partial *SU* sequences have been evaluated from naturally OPPV-infected sheep from eastern Kansas, and these *SU* sequences also had a higher percent nucleotide identity to CAEV than to MVV (Chebloune et al., 1996).

3.3. Phylogenetic analysis of SRLVs using SU

To further evaluate the differences in *SU* between Dubois and WLC1 OPPVs and other SRLVs, phylogenetic analysis was conducted. Parsimony with heuristic phylogenetic tree using phylogenetic analysis using parsimony was constructed (Fig. 1). The 16 OPPV Dubois *SU* sequences segregated into one cluster with a bootstrap value of 100. In addition, the Dubois *SU* sequences and the other OPPV *SU* sequences (S93, 85/34, and WLC1) formed one large OPPV clade with a bootstrap value of 100. *SU* sequences were significantly similar between all the OPPVs examined in this study and phylogenetically grouped together, suggesting that OPPV-infected sheep across North America are infected with closely related OPPVs. In addition, although

Table 2
Mean percent nucleotide identity in *SU* of Dubois OPPVs and WLC1 OPPV compared to WLC1 OPPV, 85/34 OPPV, S93 OPPV, CAEVs, and MVVs

	OPPV WLC1	OPPV 85/34	OPPV S93	CAEV[a]	MVV[b]
WLC1	–	91.7%	85.7%	73 ± 1.2%[c]	71.4 ± 1.2%[c]
Dubois	84.6 ± 1.4%[c]	85.4 ± 1.2%[c]	86.7 ± 1.2%[c]	74.4 ± 2.1%[c]	71.7 ± 2.0%[c]

[a] Contains CAEVs listed in Table 1.
[b] Contains MVVs listed in Table 1.
[c] Based on two standard deviations.

WLC1, S93, and 85/34 OPPV have been passaged several times prior to our acquisition (possibly >30 years in the case of 85/34) but remain similar to the Dubois OPPV field isolates in terms of *SU* sequence, this indicates that *SU* in vivo and in vitro does not change rapidly.

North American CAEVs (CAEV-63 and CAEV-CO) and French CAEVs (680, 021, 032, and 786) formed a CAEV clade based on *SU* sequences with a bootstrap value of 100. The CAEV clade and the OPPV clade formed a larger OPPV/CAEV combined group with a bootstrap value of 80 suggesting that OPPV may be more related to CAEV based on *SU* sequences. MVVs including 1514-LV1-1KS1, EV1, 1772, SA-OMVV, and KM1071 formed the MVV clade based on *SU* sequences which grouped separately from the OPPV/CAEV combined group. The finding that none of the OPPVs grouped in either the CAEV or MVV clades based on complete *SU* sequence, suggests that OPPVs may be evolving into a unique clade.

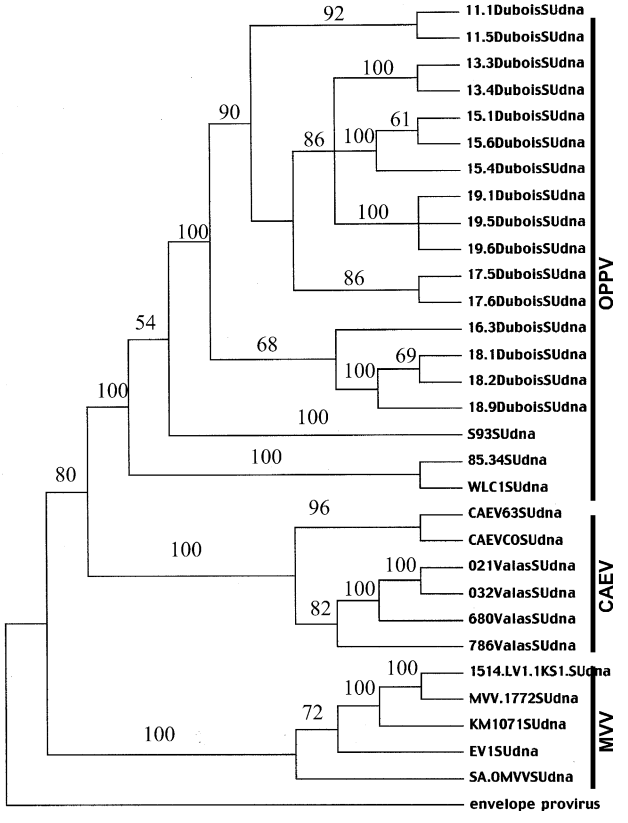

Fig. 1. Phylogenetic analysis of SRLV strains based upon *SU* sequences was generated by phylogenetic analysis using parsimony version 4.0 using parsimony with heuristic. Bootstrap confidence values were calculated based on 1000 iterations, and bovine immunodeficiency virus (BIV) *env* was used as the outgroup and is designated as envelope provirus on the bottom of the SRLV strain list.

According to our phylogenetic analysis, we observed three clades based upon complete *SU* sequences, and these clades directly corresponded to the three SRLV classifications: OPPV, CAEV, and MVV. Another study previously described five clades for SRLVs based upon both partial and complete *env* sequences (Zanoni, 1998). This previous study assigned MVVs EV1, SA-OMVV, 1514, KM1071 to clade I, North American OPPVs 85/34 and 84/28 to clade II, and North American CAEVs (CAEV-63 and CAEV-CO) and one French CAEV to clade V. Our study confirmed these clade designations and showed that complete *SU* sequences could also be used to group SRLVs according to clades.

3.4. Deduced SU amino acid sequences of SRLVs

To see if the observed nucleotide differences between OPPV, CAEV, and MVV *SU* sequences resulted in amino acid differences in *SU*, amino acid sequences were deduced from the 16 Dubois *SU* sequences, WLC1 *SU* sequence and known SRLV *SU* complete sequences found in Table 1 and CAEV 1244 (Wain-Hobson et al., 1995). The consensus sequence for all *SU* sequences (data not shown) revealed a total of 22 cysteine residues with all being completely conserved between SRLV *SU* sequences except for cysteine no. 7 in MVV-EV1 (amino acid residue 157 in consensus). In addition, 22 possible N-linked glycosylations (N-X-S/T/C) were revealed based upon the consensus sequence for SRLV, but only seven N-linked glycosylations were conserved among the SRLV *SU* amino acid sequences. The second N-linked glycosylation was conserved among the *SU* amino acid sequences of the SRLVs except Dubois 16.3.

Consistent with nucleotide sequence analysis and PAUP, WLC1 OPPV, and Dubois OPPV deduced amino acid sequences of *SU* had a significantly higher (2 S.D.) percent identity to 85/34 and S93 OPPV *SU* than CAEV or MVV *SU* sequences (Table 3). *SU* variable regions V1, V2, V3, V4, and V5 (originally designated by Valas et al., 2000) from Dubois OPPVs also showed variability. The mean percent amino acid identity in whole *SU*, V1, V2, V3, V4, and V5 was calculated for the 16 Dubois *SU* sequences against CAEV, MVV, and OPPV *SU* consensus sequences (Fig. 2). Interestingly, *SU* sequences from Dubois have a higher amino acid sequence identity in whole *SU*, V2, V3, V4, and V5 to the OPPV *SU* consensus more than MVV *SU*

Table 3
Mean percent deduced amino acid sequence identity in *SU* of Dubois OPPVs and WLC1 OPPV compared to WLC1 OPPV, 85/34 OPPV, S93 OPPV, CAEVs, and MVVs

	OPPV WLC1	OPPV 85/34	OPPV S93	CAEV[a]	MVV[b]
WLC1	–	90.9%	84.9%	73 ± 1.7%[c]	70.4 ± 5.5%[c]
Dubois	84.2 ± 1.9%[c]	86.4 ± 2.1%[c]	86.9 ± 2.4%[c]	74.0 ± 2.3[c]	69.8 ± 4.1%[c]

[a] Contains CAEVs listed in Table 1.
[b] Contains MVVs listed in Table 1.
[c] Based on two standard deviations.

or CAEV *SU* consensus sequences. However, the only region of *SU* where the Dubois *SU* had a significantly higher (2 S.D.) amino acid sequence identity to the OPPV *SU* consensus sequence over CAEV or MVV consensus sequences was V4. Since V4 contains the only known neutralizing antibody epitope in SRLVs (Skraban et al., 1999), this suggests that V4 in *SU* can be used to further group SRLVs into clades not unlike V3 in HIV *SU*. In contrast, V1 is the only region of *SU* that the Dubois OPPVs show comparable identity to OPPV and MVV consensus sequences. Interestingly, three *SU* clones from one virus (LMH 15) contained a five amino acid deletion in this V1 region, and this could have contributed to the lower percent identity to the OPPV consensus sequence.

Based on nucleotide identity, phylogenetic analysis, and deduced amino acid sequence of *SU*, Dubois and WLC1 OPPVs are more closely related to North American OPPVs than CAEV or MVV. In addition, our study and another suggest that OPPV may have evolved from CAEV more than MVV (Karr et al., 1996). This is a possibility given that lambs can be infected with CAEV by feeding them milk from infected goats (Oliver et al., 1985), and sheep can be experimentally infected with CAEV resulting in arthritis (Banks et al., 1983). Interestingly, some French MVVs isolated from two separate sheep flocks phylogenetically grouped with CAEV-CO whereas other French MVVs isolated from two different flocks phylogenetically grouped with MVV K1514 based upon a partial *SU* sequences (Leroux et al., 1997). Conversely, a goat lentivirus in Ireland more closely resembled MVVs than CAEVs based on partial *SU* sequences (Roland et al., 2002). Although none of our Dubois OPPVs grouped in the CAEV clade based on *SU* sequence, the combined observations above suggest that SRLVs are capable of cross-infecting goats and sheep. Therefore, diagnostic tests which detect all SRLVs will be highly beneficial for disease eradication. Our laboratory has recently demonstrated that a CAEV competitive-inhibition enzyme-linked immunosorbent assay (cELISA) is equally sensitive and specific in both CAEV-infected goatherds and OPPV-infected sheep flocks (Herrmann et al., 2003a, b).

In conclusion, seven new OPPV field isolates from colostrum/milk cells of Dubois sheep were isolated and analyzed based upon *SU* sequence. Based upon *SU* sequence, phylogenetic analysis of *SU*, and deduced amino acid sequence of *SU*, these new Dubois OPPVs are more closely related to North American OPPVs than to CAEVs or MVVs.

Future studies concentrating on the role of *SU* in pathogenicity and immune responses are underway. In addition,

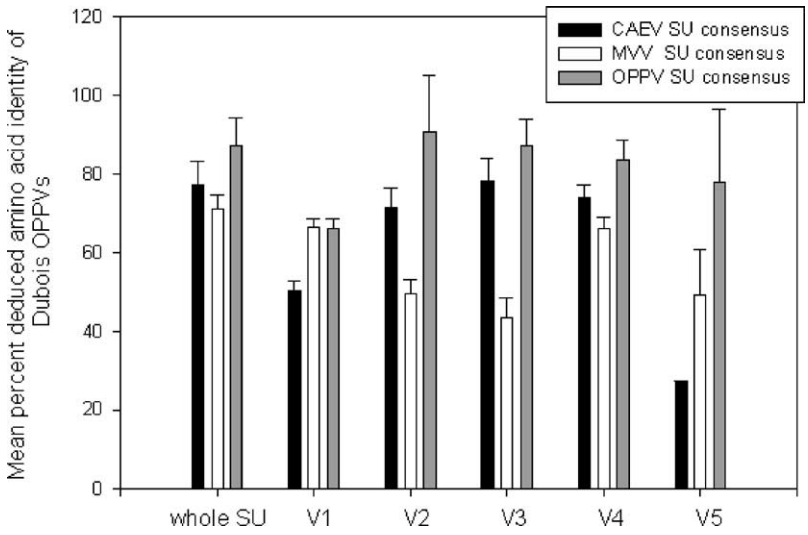

Fig. 2. Mean percent deduced amino acid identity in whole *SU* and *SU* variable regions, V1–V5, in Dubois OPPVs compared to CAEV *SU* consensus, MVV *SU* consensus, and OPPV *SU* consensus sequences. CAEV *SU* consensus sequence was constructed by aligning the CAEV *SU* sequences shown in Table 1. MVV *SU* consensus sequence was constructed by aligning the MVV *SU* sequences shown in Table 1. OPPV *SU* consensus sequence was constructed by aligning 85/34, WLC1, and S93 *SU* sequences. Error bars denote two S.D.

we are planning on evaluating *SU* amino acid sequences following ewe to lamb transmission of OPPV.

Acknowledgements

We thank Nicholas Durfee for performing cELISA tests and immunoprecipitations. We thank Emma Karel, Pete Steiner, and Duane Chandler for animal handling and colostrum/milk collection. We also thank Dr. Howard Lehmkuhl and Dr. Randall Cutlip for OPPV WLC1.

References

Andresdottir, V., Tang, X., Agnarsdottir, G., Andresson, O.S., Georgsson, G., Skraban, R., Torsteinsdottir, S., Rafnar, B., Benediktsdottir, E., Matthiasdottir, S., Arnadottir, S., Hognadottir, S., Palsson, P.A., Petursson, G., 1998. Biological and genetic differences between lung- and brain-derived isolates of maedi-visna virus. Virus Genes 16, 281–293.

Banks, K.L., Adams, D.S., McGuire, T.C., Carlson, J., 1983. Experimental infection of sheep by caprine arthritis-encephalitis virus and goats by progressive pneumonia virus. Am. J. Vet. Res. 44, 2307–2311.

Braun, M.J., Clements, J.E., Gonda, M.A., 1987. The visna virus genome: evidence for a hypervariable site in the env gene and sequence homology among lentivirus envelope proteins. J. Virol. 61, 4046–4054.

Campbell, B.J., Thompson, D.R., Williams, J.R., Campbell, S.G., Avery, R.J., 1993. Characterization of a New York ovine lentivirus isolate. J. Gen. Virol. 74, 201–210.

Chebloune, Y., Karr, B., Sheffer, D., Leung, K., Narayan, O., 1996. Variations in lentiviral gene expression in monocyte-derived macrophages from naturally infected sheep. J. Gen. Virol. 77, 2037–2051.

Cutlip, R.C., Laird, G.A., 1976. Isolation and characterization of a virus associated with progressive pneumonia (Maedi) of Sheep. Am. J. Vet. Res. 37, 1377–1382.

Cutlip, R.C., Jackson, T.A., Laird, G.A., 1977. Immunodiffusion test for ovine progressive pneumonia. Am. J. Vet. Res. 38, 1081–1084.

Cutlip, R.C., Lehmkuhl, H.D., Sacks, J.M., Weaver, A.L., 1992. Seroprevalence of ovine progressive pneumonia virus in sheep in the United States as assessed by analyses of voluntary submitted samples. Am. J. Vet. Res. 53, 976–979.

Herrmann, L.M., Cheevers, W.P., McGuire, T.C., Adams, D.S., Hutton, M.M., Gavin, W.G., Knowles, D.P., 2003a. competitive-inhibition enzyme-linked immunosorbent assay (cELISA) for detection of serum antibodies to caprine arthritis-encephalitis virus (CAEV): a diagnostic tool for successful eradication. Clin. Diagn. Lab. Immunol. 10, 267–271.

Herrmann, L.M., Cheevers, W.P., Marshall, K.L., McGuire, T.C., Hutton, M.M., Lewis, G.S., Knowles, D.P., 2003b. Detection of serum antibodies to ovine progressive pneumonia virus (OPPV) in sheep using a caprine arthritis-encephalitis virus (CAEV) competitive-inhibition enzyme-linked immunosorbent assay (ceLISA). Clin. Diagn. Lab. Immunol. 10, 862–865.

Hötzel, I., Cheevers, W.P., 2001. Host range of small-ruminant lentivirus cytopathic variants determined with a selectable caprine arthritis-encephalitis virus pseudotype system. J. Virol. 75, 7384–7391.

Jolly, P.E., Narayan, O., 1989. Evidence for interference, coinfections, and intertypic virus enhancement of infection by ovine-caprine lentiviruses. J. Virol. 63, 4682–4688.

Karr, B.M., Chebloune, Y., Leung, K., Narayan, O., 1996. Genetic characterization of two phenotypically distinct North American ovine lentiviruses and their possible origin from caprine arthritis-encephalitis virus. Virology 225, 1–10.

Kennedy, R.C., Eklund, C.M., Lopez, C., Hadlow, W.J., 1968. Isolation of a virus from the lungs of Montana sheep affected with progressive pneumonia. Virology 35, 484–484.

Knowles, D.P., Cheevers, W.P., McGuire, T.C., Brassfield, A.L., Harwood, W.G., Stem, T.A., 1991. Structure and genetic variability of envelope glycoproteins of two antigenic variants of caprine arthritis-encephalitis lentivirus. J. Virol. 65, 5744–5750.

Lairmore, M.D., Akita, G.Y., Russell, H.I., DeMartini, J.C., 1987. Replication and cytopathic effects of ovine lentivirus strains in alveolar macrophages correlate with in vivo pathogenicity. J. Virol. 61, 4038–4042.

Leroux, C., Chastang, J., Greenland, T., Mornex, J.F., 1997. Genomic heterogeneity of small ruminant lentiviruses: existence of heterogeneous populations in sheep and of the same lentiviral genotypes in sheep and goats. Arch. Virol. 142, 1125–1137.

Marsh, H., 1923. Progressive pneumonia in sheep. JAVMA 62, 458–473.

Oliver, R., Cathcart, A., McNiven, R., Poole, W., Robati, G., 1985. Infection of lambs with caprine arthritis-encephalitis virus by feeding milk from infected goats. Vet. Rec. 116, 83.

Roland, M., Mooney, J., Valas, S., Perrin, G., Mamoun, R.Z., 2002. Characterization of an Irish caprine lentivirus strain-SRLV phylogeny revisited. Virus Res. 85, 29–39.

Querat, G., Barban, V., Sauze, N., Filippi, P., Vigne, R., Russo, P., Vitu, C., 1984. Highly lytic and persistent lentiviruses naturally present in sheep with progressive pneumonia are genetically distinct. J. Virol. 52, 672–679.

Querat, G., Audoly, G., Sonigo, P., Vigne, R., 1990. Nucleotide sequence analysis of SA-OMVV, a visna-related ovine lentivirus: phylogenetic history of lentiviruses. Virology 175, 434–447.

Saltarelli, M., Querat, G., Konings, D.A.M., Vigne, R., Clements, J.E., 1990. Nucleotide sequence and transcriptional analysis of molecular clones of CAEV which generate infectious virus. Virology 179, 347–364.

Sargan, D.R., Bennet, I.D., Cousens, C., Roy, D.J., Blacklaws, B.A., Dalziel, R.G., Watt, N.J., McConnell, I., 1991. Nucleotide sequence of EV1, a British isolate of maedi-visna virus. J. Gen. Virol. 72, 1893–1903.

Skraban, R., Matthiasdottir, S., Torsteinsdottir, S., Agnarsdottir, G., Gudmundsson, B., Georgsson, G., Meloen, R.H., Andresson, O.S., Staskus, K.A., Thormar, H., Andresdottir, V., 1999. Naturally occurring mutations within 39 amino acids in the envelope glycoprotein of maedi-visna virus alter the neutralization phenotype. J. Virol. 73, 8064–8072.

Staskus, K.A., Retzel, E.F., Lewis, E.D., Wietgrefe, S.W., Silsby, J.L., St Cyr, S., Rank, J.M., Haase, A.T., Fast, D., Geiser, P.T., Harty, J.T., Kong, S.H., Cook, R., Lahti, C.J., Neufeld, T.P., Porter, T.E., Shoop, E., Zachow, K.R., 1994. Isolation of replication-competent molecular clones of visna virus. Virology 181, 228–240.

Valas, S., Benoit, C., Guinaud, C., Perrin, G., Mamoun, R.Z., 1997. North American and French Caprine arthritis-encephalitis viruses emerge from ovine maedi-visna viruses. Virology 237, 307–318.

Valas, S., Benoit, C., Baudry, C., Perrin, G., Mamoun, R.Z., 2000. Variability and immunogenicity of caprine arthritis-encephalitis virus surface glycoprotein. J. Virol. 74, 6178–6185.

Wain-Hobson, S., Sonigo, P., Guyader, M., Gazit, A., Henry, M., 1995. Erratic G → A hypermutation within a complete caprine arthritis-encephalitis virus (CAEV) provirus. Virology 209, 297–303.

Woodward, T.M., Calson, J.O., de la Concha-Bermejillo, A., DeMartini, J.C., 1995. Biological and genetic changes in ovine lentivirus strains following passage in isogeneic twin lambs. J. Acquir. Immune. Defic. Syndr. Hum. Retrovirol. 8, 124–133.

Zanoni, R.G., 1998. Phylogenetic analysis of small ruminant lentiviruses. J. Gen. Virol. 79, 1951–1961.